e maximum towards
ing spectral re-
e red.
er, New York 1973.

J. Phys. Chem.

ublication.

. 1932, 39, 164.
U.S.A. 1932, 18,

)10.
43.
on Lett., 1977,

32.
m. Chem. Soc. 1979,

. 1972, 94, 7583.

Organic Chemistry",
123-186.
try", Gold, V., Ed.,

$]_{578}$ = +114 (c =

cyclohexanone ring.
stituent at the 4-

eused if necessary.
97.
trahedron Lett.

m. Chem. Soc. 1976,

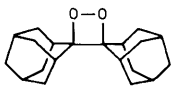
8.
, 102, 3644.
. 1980, 102, 1418.

E P I L O G U E

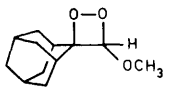
CHEMILUMINESCENCE IN ACTION; SUMMARY AND OUTLOOK

This dissertation describes a multidisciplinary study of the synthesis, properties, and applications of 1,2-dioxetanes. 1,2-Dioxetanes are energy-rich cyclic peroxides, which cleave, when heated, into two carbonyl compounds. One of the carbonyl compounds is formed in the excited state. The transition of the excited-state carbonyl to its ground state gives rise to the emission of light. Luminescence which originates from a chemical reaction is called chemiluminescence. Since the discovery of 1,2-dioxetanes, investigations in this field have concentrated on the synthesis, properties, and fundamental aspects of the chemiluminescence of these species. Thus far, no applications of these chemical sources of light are known. Our interest in this area was caused by the possibility of exploring 1,2-dioxetanes and their chemiluminescence as an analytical tool, a probe for dynamic processes, or as light source with characteristic properties.

We have investigated two types of 1,2-dioxetanes: substituted adamantylideneadamantane-1,2-dioxetanes (1) and substituted methoxymethyleneadamantane-1,2-dioxetanes (2). These two types were selected for investigations at elevated temperatures in the case of type 1, and at temperatures slightly above physiological temperatures with type 2.



1



2

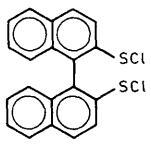
In Chapter 1 a short introduction is given on chemiluminescence in general and of 1,2-dioxetanes in particular. The recommended synthesis of 1,2-dioxetanes, of types 1 and 2, consists of the photooxygenation of an adamantane molecule with an exocyclic double bond with singlet oxygen. General aspects of singlet oxygen and adamantane are given.

Substituted adamantylideneadamantanes are the precursors for chemiluminescent labels, which are being used in analytical studies. In Chapter 2, the results are given of the investigations concerning the substitution of 3. Two

3 4

new routes were developed to introduce a halogen atom in 3. With phenylsulfenyl chloride, 3 is transformed into 4-equatorial-chloroadamantylideneadamantane 4. The same product 4 was obtained when N-chlorosuccinimide, *tert*-butylhypochlorite, or hypochlorous acid was brought in reaction with 3. When 3 was reacted with N-bromosuccinimide, the 4-eq-bromo compound (4 with Br for Cl) was formed. The unexpected results of an ionic reaction of N-halosuccinimides and 3 prompted us to extent the investigation to the halogenation of tetraalkylethylenes. This study reveals the mechanistic similarity between the halogenations of tetraalkylethylenes and the singlet-oxygen reactions with this kind of ethylenes.

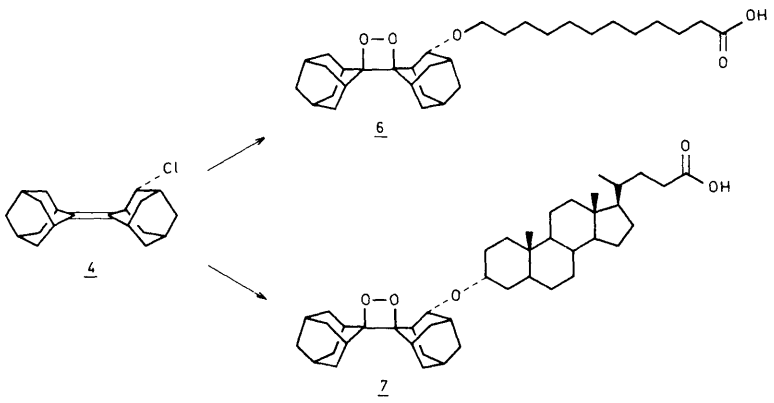
Since the starting compound 4 of our research to chemiluminescent labels is chiral, we have attempted to prepare 4 in the optically active form also. However, the reaction of 3 with optically active halogenating agents did not lead to asymmetric induction. In connection with the synthesis of one of the reagents, S-1,1'-*bis*-naphthyl-2,2'-disulfenyl chloride (S-5), we have established the absolute configurations of a series of sulfur-substituted *bis*-



S-5

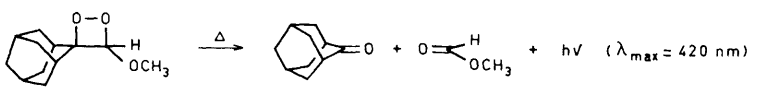
naphthyl compounds. Chapter 2 also gives the first reactions of 4 with nucleophiles in the presence of a silver salt, leading to the synthesis of a variety of substituted adamantylideneadamantanes.

In Chapter 3, the synthesis is described of two natural occurring compounds, substituted with the stable adamantylideneadamantane-1,2-dioxetane moiety (6 and 7), using the synthetic routes described in Chapter 2. Fatty acid 6 and steroid 7 are prepared in order to use these molecules as chemiluminescent labels. With the lipophilic nature of adamantane in mind, we envision the applications of 6 and 7 in the field of micelles, vesicles, and membranes. The amphiphilic character of 6 is established by its incorporation in egg lecithin vesicles.



In analytical studies with chemiluminescent labels, minute quantities of labelled biomolecules are being measured *via* the chemiluminescence, obtained on thermal activation at 200-250°C. The biomolecule can be analyzed in, for instance, immuno assays and distribution studies. Since a successful use of chemiluminescent labels strongly depends on the level of detection, we are presently building a sensitive chemiluminescence detection instrument based on photon counting. 1,2-Dioxetanes as chemiluminescent labels are important candidates to replace radioactive markers in a variety of analyses, when it will be possible to avoid absorption and energy transfer in the chemiluminescence, and the appearance of background emission. In a combined project with Drs. J.C. Hummelen we expect to realize this idea in the near future.

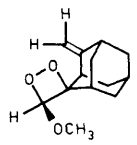
For chemiluminescence studies at temperatures below 70°C, we have developed a new type of 1,2-dioxetane 2. Chapter 4 describes the synthesis, properties, and chemiluminescence of methoxymethyleneadamantane-1,2-dioxetane (2), as well as analogues of 2. The scope and limitations of the substitution of 1,2-dioxetane 2 are studied. Variations have been applied at the 4- and 5-adamantane positions and the methoxy group of 2 is replaced by other alkoxy groups. The fragmentation of 2 furnished adamantanone and methyl formate, and bright blue chemiluminescence. The emission maximum at 420 nm indicates that the chemiluminescence is due to adamantanone fluorescence only; no phosphorescence or methyl-formate emission is observed. The reaction rates for the decomposition of 2 (k_1 = 10·10^{-5} sec^{-1} at 59°C) and the activation parameter of 2 (E_a = 26 ± 2 kcal/mole) are determined in toluene. The ratio of singlet *versus* triplet excited-state formation is established as $\phi_S:\phi_T$ = 1:4.



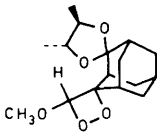
The chance observation, that enol ethers give chemiluminescence when heated in the air at 230°C, has initiated the research into the thermal oxidation of electron-rich olefins. The results of this study indicate the remarkable similarity between the reaction of $^1O_2$ at ambient temperatures and $^3O_2$ at elevated temperatures with enol ethers and tetraalkylethylenes. In the former reaction, 1,2-dioxetanes are isolated and in the latter reaction, 1,2-dioxetanes are proposed as intermediates in the chemiluminescent autooxidations.

A synthetic route to a fatty acid, that is substituted with the methoxymethyleneadamantane-1,2-dioxetane moiety, is indicated in Chapter 4. This molecule is a potential probe for the study of dynamic processes, since it will chemiluminesce already at temperatures slightly above physiological temperatures. The nature of the chemiluminescence of 2 is dependent on the environment of 2; however, with the limited number of data available so far, a clear relation between these two has not been established. Another application of these kinds of molecules could be the formation of excited-state compounds in biological material, without using incident light. With the synthetic foundations described in this Chapter 4, these applications may be realized in the near future.

Optically active 1,2-dioxetanes are the subject of Chapter 5. The enol ether 1,2-dioxetanes 8 and 9 are prepared. The degree of circular polarization of the chemiluminescence of these 1,2-dioxetanes is identical with the degree of circular polarization of the fluorescence of the corresponding ketones. The



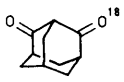
8



9

explanation for this result is the similarity of the geometry of the ketones at the moment of emission whether the ketone reaches its excited state by photoexcitation or by chemiexcitation. The difference in stability of 8 and its isomers reflects the influence of electronic and steric factors in the fragmentation. In connection with our study of chemiluminescence we have found that the bioluminescence of the firefly larvae is circularly polarized. Most remarkable is the opposite sign of this circular polarization for the two lanterns of the larvae. An explanation is given in terms of a macroscopic mirror-image relationship.

In Chapter 5 the first synthesis of an optically active compound, which owes its chirality to the presence of an excited state, is described. With a lifetime of a few nanoseconds a 1,3-diketone with an enantiomeric excess of 69% is prepared. The detection of the optical activity was unsuccessful, due to rapid energy transfer, which results in racemization. This kind of experiments can be used in the measurements of energy-transfer rates. Using the same reaction sequence with in the last step $^{18}O_2$ instead of $^{16}O_2$, we have prepared (S)-2,4-adamantanedione-4-^{18}O (10) with an enantiomeric excess of 69% and an isotopic purity of 65%. The chiroptical properties of the 1,3-diketone 10,



10

which owes its chirality solely to oxygen-isotope substitution, are recorded. The reaction of singlet oxygen-18 with electron-rich enol ethers represents a new and nonaqueous route to oxygen-18 carbonyl compounds. The intermediate in this sequence is an oxygen-18 1,2-dioxetane.